

Claim Amendments

1. (Currently amended) A replicase complex comprising an HCV NS5B protein, a linear nucleic acid template and a complementary nucleic acid primer which is annealed to the 3' terminus of the template, wherein the template is at least ~~[[three]]~~ five nucleotides and the primer is two or three nucleotides, and the template and primer do not form a stable duplex in solution in the absence of the HCV NS5B protein.
2. (Currently amended) The replicase complex according to ~~[[of]]~~ claim ~~[[1]]~~ 23, wherein the primer sequence is selected from GG, GC, CG, CC, GU, UG, CA, and AC.
3. (Currently amended) The replicase complex according to ~~[[of]]~~ claim ~~[[1]]~~ 23, wherein the base of the first nucleotide of the primer is a guanine.
4. (Currently amended) The replicase complex according to ~~[[of]]~~ claim ~~[[1]]~~ 23, which is in contact with an inhibitory compound of the HCV NS5B protein.
5. (Currently amended) An assay system for detecting HCV replicase activity comprising
an enzymatically active amount of HCV NS5B protein;
an RNA template which comprises at least five nucleotides; ~~[[and]]~~

an RNA primer which is complementary to the 3' terminus of the template and comprises two or three nucleotides, wherein the template and primer do not form stable duplex in solution in the absence of the NS5B protein, ATP, GTP, CTP, and UTP nucleoside nucleotide triphosphates (NTPs), wherein only one of the NTPs or the primer is radiolabeled [[.]]; and an assay buffer that permits replication activity of the NS5B protein.

6. (Currently amended) The assay system according to [[of]] claim 5, wherein the base of the first nucleotide of the primer is a guanine.

7. (Currently amended) The assay system according to [[of]] claim 5, wherein the NS5B protein is in contact with an inhibitory compound of the NS5B protein.

8. (Currently amended) The assay system according to [[of]] claim 5, wherein the NS5B protein is a soluble enzymatically active NS5B protein ~~and has enzymatic activity when expressed in Escherichia coli.~~

9. (Currently amended) The assay system according to [[of]] claim 5, wherein the RNA template lacks any stable secondary structure at the 3' terminus.

10. (Currently amended) The assay system according to ~~[[of]]~~ claim 5, wherein the ~~radiolabel~~ radiolabeled NTP or primer comprises ~~[[is]]~~ a phosphate isotope.

11. (Currently amended) The assay system according to ~~[[of]]~~ claim ~~[[5]]~~ 10, wherein the radiolabeled NTP is an α -³³P-NTP which hydrogen bonds to a nucleotide of the template.

12. (Currently amended) The assay system according to ~~[[of]]~~ claim 5, wherein

the assay buffer comprises 50 mM HEPES (pH 7.3), 10 mM β -mercaptoethanol, 50 mM NaCl, and 5 mM MgCl₂~~[[,]]~~;

the template RNA is 5 μ M~~[[,]]~~;

the primer is 10 μ M~~[[,]]~~;

the HCV NS5B protein is 3 to 5 μ M~~[[,]]~~; and

the NTP substrate is 100 μ M.

13. (Currently amended) A method for detecting HCV replicase activity, comprising

(a) providing an assay system comprising

an enzymatically active amount of HCV NS5B protein;

an RNA template which comprises at least five nucleotides;

an RNA primer which is complementary to the 3' terminus of the template and comprises two or three nucleotides, wherein the template

and primer do not form stable duplex in solution in the absence of the NS5B protein.

ATP, GTP, CTP, and UTP nucleotide triphosphates (NTPs), wherein one of the NTPs or the primer is radiolabeled; and an assay buffer that permits replication activity of the NS5B protein;

(b) incubating said assay system under conditions that permit NS5B polymerase activity; and

(c) detecting a nucleic acid synthesized by [[an]] the HCV NS5B protein on a linear RNA template which comprises at least three nucleotides and an RNA primer which comprises two or three nucleotides, wherein the primer basepairs to the 3' end of the template and the RNA template and the primer do not form a stable duplex in the absence of HCV NS5B protein, in the presence of ATP, GTP, CTP, or UTP (NTPs), wherein only one of the NTPs is radiolabeled, and an assay buffer that supports replication activity of NS5B.

14. (Currently amended) The method according to claim 13, wherein said detecting the nucleic acid synthesized by NS5B comprises evaluating an autoradiograph of reaction products separated by gel electrophoresis.

15. (Currently amended) The method according to claim 13, wherein the NS5B protein is a soluble NS5B protein expressed in Escherichia coli.

16. (Original) The method according to claim 13, wherein the base of the first nucleotide of the primer is a guanine.

17. (Currently amended) The method according to claim 13, wherein the ~~radiolabel is~~ radiolabeled NTP or primer comprises a phosphate isotope.

18. (Currently amended) The method according to claim ~~[[13]]~~ 17, wherein the radiolabeled NTP is an α -³³P-NTP which hydrogen bonds to a nucleotide of the template.

19. (Currently amended) The method according to claim 13, wherein the assay buffer comprises 50 mM HEPES (pH 7.3), 10 mM β -mecaptoethanol, 50 mM NaCl, and 5 mM MgCl₂~~[[.]]~~;

the template RNA is 5 μ M~~[[.]]~~;

the primer is 10 μ M~~[[.]]~~;

the HCV NS5B protein is 3-5 μ M; ~~[[and]]~~

the NTP is 100 μ M; and

the assay is performed at 30°C.

20. (New) The method according to claim 13, wherein said assay system further comprises a candidate inhibitory compound.

21. (New) The method according to claim 20, wherein said method is a high throughput screen.

22. (New) The method according to claim 20, wherein said candidate inhibitory compound directly interferes with the replicase activity of the NS5B protein.

23. (New) The replicase complex of claim 1, wherein the nucleic acid template and the nucleic acid primer are RNA.

24. (New) The assay system according to claim 5, wherein said HCV NS5B protein, RNA template and RNA primer form a replicase complex.